Comparison between human and porcine thromboelastograph parameters in response to ex-vivo changes to platelets, plasma, and red blood cells

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In the acute care setting, both the tracings and numeric outputs (R time, angle, and MA) of thrombelastography (TEG) may be used to inform treatment decisions. The objective was to determine the sensitivity of TEG to isolated changes in platelet count, hematocrit and fibrinogen concentration in human blood. As pigs have a similar coagulation system, we also compared the responses of the pig blood. Eight volunteers (>18 years of age, no anticoagulation or nonsteroidal anti-inflammatory therapy, not pregnant) were enrolled into this study. Four female anesthetized donor pigs were instrumented percutaneously with a catheter for blood collection. All blood was collected into sodium citrate. The concentration of each component (platelets, fibrinogen, and red blood cells) was changed while keeping the other components constant by use of centrifugation or preparation of each individual's plasma into platelet poor plasma, platelet rich plasma, cryoprecipitate, purified washed platelets, and packed red blood cells as appropriate. TEG (Haemoscope) analysis was performed and compared with the patients' whole blood diluted with lactated Ringer's solution. We demonstrated that the major factor affecting the MA and angle was the platelet count. In fact, reducing platelets alone resulted in

TEG profiles and parameters that were similar to lactated Ringer's dilution profiles. Swine blood responses were parallel to that of human blood, although there were offsets especially of TEG-R and angle that confirmed that the swine are hypercoagulable compared with humans. Superficially similar TEG tracing patterns can be produced by divergent mechanisms associated with altered concentrations of blood components. *Blood Coagul Fibrinolysis* 24:818–829 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

Hemorrhage remains the major cause of death in potentially survivable injuries, yet mortality has not improved significantly over the past 30 years [1]. Strategies of hypotensive resuscitation [2], restriction of crystalloids [3], and more balanced administration of blood components – adding plasma and platelets to red blood cells (RBCs) [4,5] – as well as resuscitation algorithms [6], have recently been implemented for the severely injured.

The use of thromboelastography (TEG) in the trauma bay has been proposed as a technique that can be used to develop goal-directed resuscitation therapy with blood components [7,8]. The TEG is a whole blood assay that measures the changes in viscoelasticity as the blood clots after activation of coagulation with an accelerant, either kaolin or tissue factor or both as in the case of 'rapid' TEG. The TEG tracing represents the cell-based theory of coagulation: clot initiation (R-time), clot propagation (α-angle), and clot strength (MA) [9]. In addition, the first derivative of the upper trace has been correlated to

thrombin generation and the area under the curve reflects the final clot strength [10]. The developers of the TEG state that the shape of the clotting curve gives an indication of hypocoagulation or hypercoagulation and status of fibrinolysis, either normal, primary or secondary [11]. There are claims in the trauma literature that the prolongation of the R-time reflects clotting factor deficiency or dilution, prolongation of K-time and a smaller α-angle reflects low fibrinogen and platelets, and low MA reflects the platelet and fibrinogen contribution to clot strength [12–15].

However, recent publications describing manipulations of various blood components made *in vitro* have not borne out the claims that specific deficits can be determined by the TEG. For example, a study by Nielsen *et al.* [16] measured the TEG response of various plasmas, which were deficient in only one factor each. For example, R-time was affected by deficiencies of each one of the factors except FXIII. α-Angle was reduced with deficiencies of FII, FVII, FX, and FXII. The MA was

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reduced with deficiencies of FII, FVII, FX, FXII, and FXIII. The authors also showed that fibrinogen affects all three: R-time, α-angle, and MA. A limitation of this study was that platelets and red blood cells were not present as they would be in whole blood.

Bochsen *et al.* [17] compared TEG responses with two levels of platelets, 0 and 200×10^9 per l, as well as three levels of hematocrit (0, 15, and 29%) and plasma concentration (0, 25, and 50%). They found that increasing hematocrit reduced the α-angle and MA both with and without platelets. The presence of platelets increased the α-angle and MA compared with no platelets. Their results are difficult to interpret because they altered hematocrit and plasma concentration at the same time.

In an in-vitro model of thrombocytopenia, Larsen *et al.* studied the effect of isolated changes in platelet count on the rotational thromboelastometry (ROTEM) parameters using platelet-rich plasma and platelet-poor plasma recombined with red blood cells at a normal hematocrit [18]. They reported a logarithmic reduction in maximum velocity with the reduction in platelet count from 307 to 16×10^9 per l.

To mimic the dilution that occurs in resuscitated trauma patients, Darlington *et al.* [19] measured changes in TEG parameters using tissue factor as an accelerant in response to 0–90% dilution with physiological saline (0.9% NaCl). The TEG parameters showed logarithmic changes with dilution in all of the parameters. As all three components of the blood was equally diluted (i.e. hematocrit, fibrinogen, and platelets), it is not possible to determine how the changes in the TEG parameters were affected by each component.

Using human blood from eight healthy individuals, our goal was to manipulate each blood component (hematocrit, fibrinogen, and platelets) independently while keeping the other two components constant. In this way, we were able to determine which TEG parameter was affected the most by each component, with the idea that if the TEG can be used to reflect which blood component should be administered to a trauma patient, specific predictable changes should be able to be detected for each component. As pigs have been used to develop models of trauma [20] and have a similar coagulation system [21] we set out to compare the responses of the pig blood as well.

Methods

The methodology for these in-vitro studies was developed for the human samples and then applied to pig samples. The four groups were lactated Ringer's-diluted blood (LR DIL), platelet-adjusted whole blood (PAWB), hematocrit-adjusted whole blood (HAWB), and fibrinogen-adjusted whole blood (FAWB) (Table 1).

Human blood collection procedure

This study was conducted under a protocol reviewed and approved by the US Army Medical Research and Materiel Command Institutional Review Board and in accordance with the approved protocol. Whole blood was collected from eight normal healthy volunteers (four men, four women; age 33 ± 2 years; range: 26–42) after signing an informed consent. Exclusion criteria were: ongoing therapeutic anticoagulation therapy, use of over-the-counter drugs such as aspirin, ibuprofen, herbal products, or nonsteroidal anti-inflammatory drugs within 7 days, and pregnancy. Each participant was sampled four times. Blood was collected by venipuncture of the medial cubital vein using a 21 ga. × 0.75 in. needle winged blood collection set (Becton–Dickinson, Franklin Lakes, New Jersey, USA). Ten to 15 tubes (4.5 ml blue top 3.2% Na citrate, Becton-Dickinson) of blood were collected, depending on which procedure was performed on that

Table 1 Each component's adjustments for the four groups

SAMPLE	COMPONENT (units)	PAWB	HAWB	FAWB	LR DIL
NWB	PLT# ($\times 10^9$/l WB)	PTS PLT	PTS PLT	PTS PLT	PTS PLT
A	PLT# ($\times 10^9$/l WB)	200	50	50	200
B	PLT# ($\times 10^9$/l WB)	100	50	50	25% diluted w LR
C	PLT# ($\times 10^9$/l WB)	50	50	50	50% "
D	PLT# ($\times 10^9$/l WB)	25	50	50	75% "
E	PLT# ($\times 10^9$/l WB)	PPP	50	50	87.5% "
NWB	HCT (%)	PTS HCT	PTS HCT	PTS HCT	PTS HCT
A	HCT (%)	PTS HCT	40	40	40
B	HCT (%)	PTS HCT	30	40	25% diluted w LR
C	HCT (%)	PTS HCT	20	40	50% "
D	HCT (%)	PTS HCT	10	40	75% "
E	HCT (%)	PTS HCT	0	40	87.5% "
NWB	[FIB] (mg/dl WB)	PTS [FIB]	PTS [FIB]	PTS [FIB]	PTS [FIB]
A	[FIB] (mg/dl WB)	PTS [FIB]	PTS [FIB]	CR	PTS [FIB]
B	[FIB] (mg/dl WB)	PTS [FIB]	PTS [FIB]	50% diluted w CP	25% diluted w LR
C	[FIB] (mg/dl WB)	PTS [FIB]	PTS [FIB]	75% "	50% "
D	[FIB] (mg/dl WB)	PTS [FIB]	PTS [FIB]	87.5% "	75% "
E	[FIB] (mg/dl WB)	PTS [FIB]	PTS [FIB]	CP	87.5% "

[FIB], fibrinogen concentration; CP, cryo poor; CR, cryo rich; FAWB, fibrinogen-adjusted whole blood; HAWB, hematocrit-adjusted whole blood; HCT, hematocrit; LR DIL, LR dilution; NWB, normal whole blood; PAWB, platelet-adjusted whole blood; PLT, platelet; PTS, patient's; WB, whole blood.

day, and allowed to rest upright in a test tube rack for 30 min at room temperature. A baseline sample was also collected for hematocrit and platelet count determination.

Pig blood collection procedure

This study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. This study has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals.

Yorkshire-cross female pigs weighing 53.7 ± 1.5 kg were obtained from Midwest Swine Research (Gibbon, Minnesota, USA). Each pig was fasted approximately 12–18 h prior to the blood draw procedure with water available *ad lib*. On the day of the procedure, the pigs were injected with glycopyrrolate (0.01 mg/kg) and tiletamine–zolazepam (8 mg/kg) intramuscular, for secretion control and sedation, respectively. Anesthesia was induced via a facemask with ~5% isoflurane in 100% oxygen and the animals were intubated. During the percutaneous procedure, anesthesia was maintained with 1–3% isoflurane in 100% oxygen using a ventilator and monitor (Draeger Medical Apollo Gas anesthesia system w/ isoflurane vaporizer and Infinity Explorer Monitoring System; Telford, Pennsylvania, USA). Sterile procedure was followed using ultrasound (Sonocite 180; SonoCite, Inc., Bothell, Washington, USA) to guide access to the femoral vein. The vein was cannulated (SI-09700, 7 Fr. Side-port/ Percutaneous Sheath Catheter Introducer Set; Arrow International, Reading, Pennsylvania, USA). Blood (150 ml) was collected in syringes (30 ml capacity, Becton–Dickinson) and transferred into 33 tubes (4.5 ml blue top 3.2% Na citrate) and the tubes were allowed to rest upright in a test tube rack for 30 min at room temperature. A baseline sample was also collected for hematocrit and platelet count determination.

As the results had such small standard deviations for the humans, a power analysis demonstrated that only $n = 4$ were needed to be able to detect significant changes with the pig data.

Blood component procedure

From this point forward, the same procedures were used on both human and pig blood. An overview of each component's target adjustments is shown in Table 1. The resulting levels of each of the blood components (hematocrit, platelets, and fibrinogen) in the four treatments (lactated Ringer's, HAWB, PAWB, and FAWB) are shown in Fig. 1. The platelet count was measured for each step (Z2 Particle Counter; Beckman Coulter, Miami, Florida, USA). Each of the components was pooled into a 50-ml polypropylene conical tube (Corning

Fig. 1
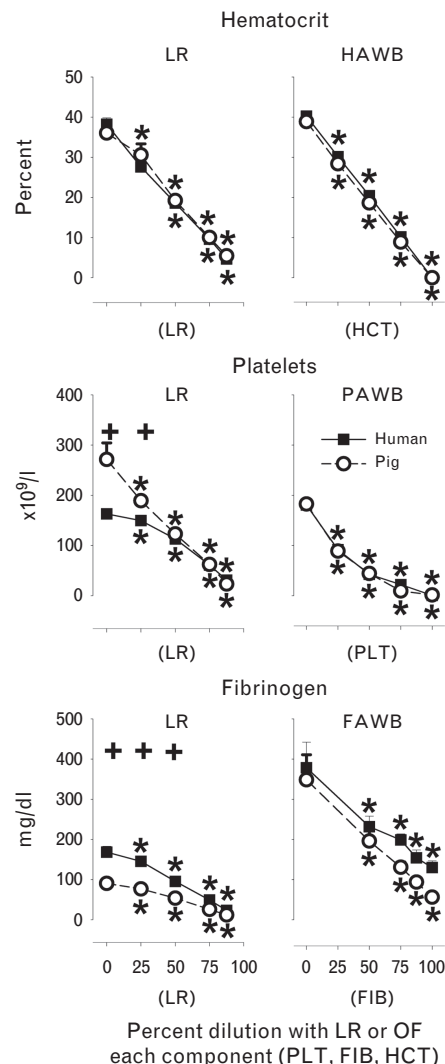
Comparison of each of the blood components [hematocrit (HCT), platelets (PLT), and fibrinogen (FIB)] between the lactated Ringer's (LR) and the corresponding HAWB, PAWB, and FAWB treatments. $+P < 0.05$ for difference between pig ($n = 4$) and human ($n = 8$) at the dilution over which the symbol is plotted. $*P < 0.05$ for difference between 0% and the dilution indicated for each.

Inc, Corning, New York, USA), kept tightly capped and upright at room temperature until used.

Platelet rich plasma

The tubes of citrated blood were centrifuged (Jouan CR74i; Thermo Electron Corp., Chateau-Gontier, France) at $150 \times g$ for 10 min.

Platelet poor plasma

The tubes of citrated blood with platelet rich plasma (PRP) removed were re-centrifuged (Sorvall RT1; Thermo Electron Corp., Chateau-Gontier, France) at $2000 \times g$ for 10 min.

Red blood cells

After removal of the platelet poor plasma (PPP), the buffy coat was suctioned off the RBCs remaining in the citrated tubes to remove the platelets and resulted in what was considered to be a 100% RBC solution.

Cryoprecipitate

Cryoprecipitate (Cryo) was made on the same day that the blood was collected from the participants after PPP, PRP, and RBCs were made as described above. The PPP was transferred to a 50-ml polypropylene conical tube and frozen at $-80°C$ for 10–30 min. The frozen PPP was placed into an iced water bath until the sample formed a slush (approximately 60 min). The sample was centrifuged at $2000 \times g$ for 10 min at $4°C$. Approximately 90% of the Cryo-poor platelet poor plasma supernatant (CP PPP) was removed from the pellet of Cryo-rich plasma precipitate and transferred to a 50-ml polypropylene conical tube without disturbing the pellet. The Cryo-rich plasma precipitate pellet with 10% of the PPP was warmed to $37°C$ to dissolve the precipitate. The CP PPP and CR PPP were assayed for fibrinogen concentration (STart 4; Diagnostica Stago, Inc., Parsippany, New Jersey, USA).

Washed platelets (used only in the fibrinogen-adjusted whole blood group): reagents

Sodium chloride, potassium chloride, magnesium chloride, sodium phosphate monobasic, sodium bicarbonate, D(+)glucose, HEPES sodium salt, prostaglandin I_2 sodium salt (PGI_2), apyrase, bovine serum albumin (BSA), trisodium citrate, and citric acid were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Missouri, USA). Phosphate-buffered saline (PBS) was purchased from Fisher Diagnostics (a division of Thermo Scientific, Middletown, Virginia, USA).

Stock solutions

Stock solutions of modified Tyrodes buffer (2.73 mol/l NaCl, 53.6 mmol/l KCl, 238 mmol/l $NaHCO_3$, 8.6 mmol/l NaH_2PO_4), Magnesium chloride (0.1 mol/l), HEPES buffer (0.5 mol/l) and modified acidified citrate dextrose (ACD, 30.8 mmol/l trisodium citrate, 190 mmol/l citric acid, 316 mmol/l dextrose) were made and kept refrigerated for several weeks. Bovine serum albumin stock (BSA, 20% w/v in distilled water), Apyrase stock (200 U/ml in distilled water), and prostaglandin I_2 stock (20 mg/ml in 100% ethanol) were frozen in 10 µl aliquots.

Working buffers

The modified Tyrode HEPES albumin buffer (MTAB, containing 137 mmol/l NaCl, 2.7 mmol/l KCl, 1.0 mmol/l $MgCl_2$, 0.43 mmol/l NaH_2PO_4, 12 mmol/l $NaHCO_3$, 5.5 mmol/l D(+) glucose, 5 mmol/l HEPES, and 0.35% BSA, adjusted to pH 7.35) was made fresh daily from stock solutions and diluted with distilled water.

Platelet washing procedure

Ten millilitres of PRP were acidified to a pH of 6.5 with ACD. Apyrase (0.02 U/ml final concentration) and PGI_2 (1 µmol/l) were added to the acidified PRP. The tubes were centrifuged at $750 \times g$ for 10 min at room temperature, the plasma was aspirated, and the platelet pellet in each tube was resuspended in 10 ml MTAB. Apyrase (0.01 U/ml) and PGI_2 (0.5 µmol/l) were added. The washed platelets in MTAB were divided into a 4-ml aliquot used to make cryo-rich PRP (CR PRP) and a 6-ml aliquot, which was used to make cryo-poor PRP (CP PRP). The tubes were re-centrifuged at $750 \times g$ for 10 min; the supernatants were discarded. The platelet pellets were resuspended in 1.0 ml of either cryo-rich or cryo-poor PPP and the platelet count was measured in each (Z2 Particle Counter; Beckman Coulter, Miami, Florida, USA). These tubes of cryo-rich PRP and cryo-poor PRP were then combined appropriately to result in a platelet count of 92×10^9 per l in either 2.5 ml of cryo-rich PRP or 3.5 ml of cryo-poor PRP.

Sample analysis procedures

For each of the four groups, five dilutions were made (Table 1). Aliquots of each dilution (A–E), as well as the baseline blood samples (normal whole blood, NWB) were analyzed for whole blood fibrinogen, complete blood count (CBC, Cell-Dyn 3700CS hematology analyser; Abbott Laboratories, Abbott Park, Illinois, USA), and thromboelastography (TEG, Haemoscope 5000; Haemonetics Corp., Braintree, Massachusetts, USA). For the TEG samples, a 1-ml aliquot was pipetted into a kaolin tube to initiate coagulation and 340-µl samples were pipetted into TEG cups with 20 µl of calcium chloride (0.2 mmol/l). Prior to TEG assaying, the five dilutions of each type were allowed to sit at room temperature for 30 min, and then incubated in a $37°C$ water bath for 30 min. The TEG samples were measured at $37°C$. Standard TEG-derived parameters (Fig. 2, top panel) were measured as well as the first derivative of the upper trace of the TEG curve (Fig. 2, middle panel). The samples were run in duplicate.

Lactated ringer's dilution procedure

Four serial dilutions of the initial whole blood sample using citrated lactated Ringer's (LR) as the diluent were made to result in a target level of hematocrit of 30, 20, 10 and 5% and their corresponding whole blood platelet count target of 90, 60, 30, and 15×10^9 per l.

Platelet adjusted whole blood procedure

A stock solution of platelet poor whole blood (PPWB) was made by adding PPP to 100% RBCs to obtain the participant's baseline hematocrit level. The patient's normal whole blood (NWB) and the stock of PPWB were

Fig. 2

Thromboelastograph (TEG)

TEG velocity curve
(first derivative of top of TEG curve
after conversion to G [dyne/cm²])

Calibrated automated thrombinoscope (CAT)
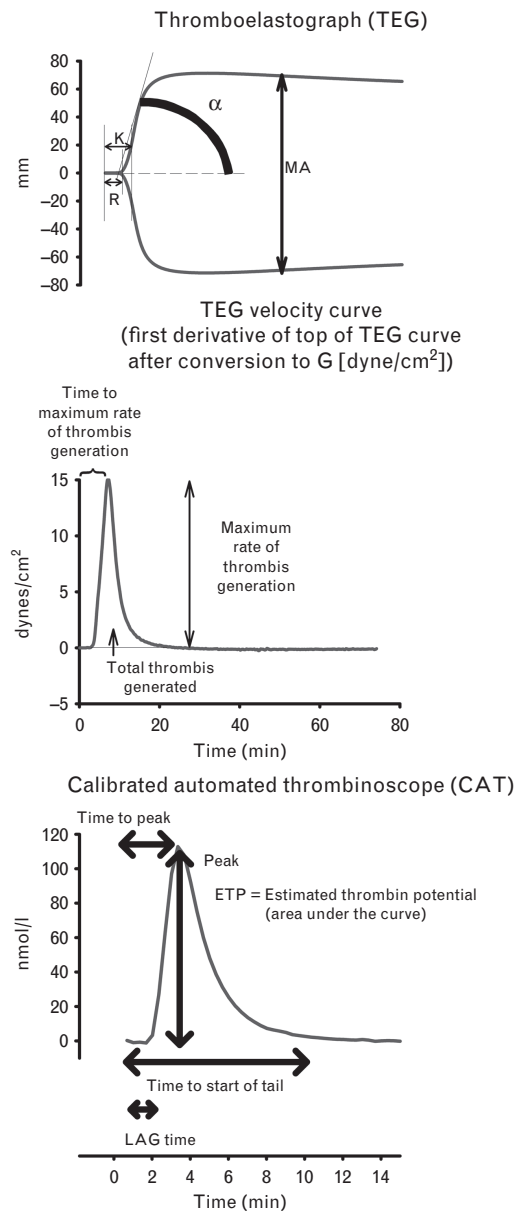
Description of parameters derived from the TEG fork graph, velocity graph, and the computerized activated thrombinoscope (CAT) graph. Top panel: TEG variables of R (clot initiation time, minutes), K (clotting time in minutes to a fixed 20-mm of clot strength), angle (degrees, kinetics of clot development), MA (mm, maximum amplitude, maximum clot strength), and G (shear elastic modulus, dynes/cm², derived from MA by the formula $G = [5000 \times MA]/[100 - MA]$). Middle panel: The velocity graph variables are TEG-TG (positive area under the first derivative of the top fork coordinates after conversion to G, divided by 5 s to obtain thrombus generation in units of dynes/cm²), TEG-MRTG (maximum rate of thrombus generation is the maximum peak height in units of dynes/cm²), TEG-TMRTG (time to maximum rate of thrombus generation in minutes).

combined in appropriate amounts to obtain a platelet count of 200×10^9 per l, followed by serial dilutions to reach whole blood platelet count targets of 100, 50, and 25×10^9 per l, along with PPP.

Hematocrit-adjusted whole blood procedure
Dilutions of HAWB were made so that the hematocrits were at five levels (40, 30, 20, 10, and 0%). The fibrinogen levels in each tube were fixed at one level determined by each patient's normal levels as occurred in the PPP and PRP. The whole blood platelet count in each tube was adjusted to be equal to fixed level of 50×10^9 per l. Varying volumes of the 100% RBC were added to the samples with the remaining volume made up by adding PBS in appropriate volumes.

Fibrinogen adjusted whole blood procedure
Dilutions of FAWB were made so that the fibrinogen concentrations were at five levels, which were dependent on each patient's levels in their cryo-rich plasma and cryo-poor plasma (cryo-rich whole blood, 50, 75, and 87.5%, and cryo-poor whole blood). The hematocrits were adjusted to 40% in each tube by adding an appropriate amount of 100% RBC. The whole blood platelet count in each tube was adjusted to be equal to final level of 50×10^9 per l by using the cryo-rich PRP and the cryo-poor PRP.

Calibrated automated thrombogram
Calibrated automated thrombin generation was measured using PRP with the platelet count adjusted to 200, 100, 50, and 25×10^9 per l using autologous PPP (Thrombinoscope, Thermo Electron Fluoroskan Ascent; ThermoLabsystems, Helsinki, Finland). The reagents for measuring thrombin generation in PRP are PRP Reagent (1.0 pmol/l TF final concentration, and minimal phospholipids), Thrombin Calibrator, and FluCa Reagent kit (HEPES buffer with calcium chloride and fluorogenic substrate solubilized in DMSO) were used per manufacturer instructions. The samples were run in triplicate. Run time was preset for 1 h. See Fig. 2, bottom panel, for a description of calibrated automated thrombogram (CAT) parameters.

Statistical analysis
For the data compared with the percentage dilution, a two-way repeated measures analysis of variance, with species as one factor and the percentage dilution as a repeated factor, was performed using the general linear model (SigmaPlot, Version 11.0; Systat Software, Inc., Chicago, Illinois, USA). If there was a significant interaction effect, a one-way repeated measures analysis was performed for each species and a posthoc analysis was performed (Holm-Sidak method) for each TEG parameter compared with the zero dilution level.

The TEG velocity and CAT peak height slopes derived from linear regression, as well as the CAT versus TEG correlations were analyzed using analysis of covariance (Version 9.2; SAS Institute Inc., Cary, North Carolina, USA).

The TEG parameter data were $\log_{10}$ transformed and a regression analysis using a two-order polynomial was used to fit the TEG parameters against levels of platelets, hematocrit, or fibrinogen. Interaction effects associated with species (human versus pig) were tested for the quadratic, linear and intercept components of the model. *P*-values less than 0.05 were considered significant. (JMP, Version 9.0; SAS Institute Inc.) The statistical significance is given in table form adjacent to the graphical results of each of the comparisons. Results are presented as means $\pm$ SD.

Results

As shown in Fig. 1 for the lactated Ringer's group, the dilution of the platelets and fibrinogen levels were determined by each of the patient's endogenous baseline levels, while the starting hematocrit was set at 40% for both the human and pig. Thus, while there were significant reductions from baseline, there were also significant differences between humans and pigs in the platelet count and fibrinogen concentration for the lactated Ringer's treatment at baseline (Fig. 1). There was no difference between human and pig for the PAWB, HAWB, and FAWB treatments since the platelets and hematocrit were targeted at specific levels for both the human and pig, while the fibrinogen levels were determined by each of the patient's endogenous baseline levels or for the cryo-rich level in FAWB.

Thrombin generation

The TEG responses were compared with CAT responses for the different levels of platelets. The TEG and CAT parameters are defined in Fig. 2. The human CAT results are shown in the top left panel (Fig. 3); the amount of thrombin generated was related to the platelet count, as expected. There was also a relationship between the porcine thrombin generation and platelet count, but the peak height (top right panel, Fig. 3) and some of the other calculated parameters (Fig. 4) were considerably lower in the pig than the human. In the CAT assay, the fluorescent substrate used in the technique is optimized for human blood, which may explain why pig blood yielded consistently lower peaks. The TEG velocity (center panel, Fig. 3) was compared with the CAT peak height, and a significant correlation ($P < 0.001$) between TEG-MRTG and CAT peak height was shown for each species (bottom panel, Fig. 3). Therefore, the TEG MRTG parameters were used in lieu of the CAT assay to estimate thrombin generation for the remainder of the component manipulation studies due to concerns about the species specificity of the CAT assay.

Thrombelastography responses

In comparing the TEG parameters between human and pig with respect to percentage of dilution of the different components (data not shown), the responses were parallel within each manipulated component, but there were significant differences between the species. Generally, the pig results were hypercoagulable (shorter R, K, the TMRTG, and higher clot strength and velocity parameters) for comparable dilutions compared with humans. Because there were significant differences in the baseline values of the platelets and fibrinogen between pig and human when expressed as a percentage dilution, we analyzed each of the TEG parameters with respect to the concentrations of the three manipulated components and compared each of the individual components with the TEG responses of lactated Ringer's dilution (Figs 5–7, see below).

Platelets: lactated Ringer's and platelet adjusted whole blood procedure

All TEG parameters (defined in Fig. 2) showed a dose-related change with lactated Ringer's dilution when plotted against platelet count (Fig. 5). Overall, the response to manipulation of the platelets alone (PAWB) was similar to that with lactated Ringer's dilution for the TEG-K, TEG-angle, TEG-MA, TEG-G, and TEG-TG parameters. In contrast, there was no significant dose-related response in TEG-R and TEG-TMRTG parameters to PAWB, which remained horizontal with the change in PLT.

The graph of many of the responses of the TEG parameters were remarkably similar with the manipulation of the platelets alone compared with simultaneous dilution of all three blood components in lactated Ringer's treatment, although the design of our study did not allow direct statistical comparison. In order to make that comparison, the manipulated component should have been adjusted to the same level for both species rather than using dilution from endogenous baseline values. The steepest reduction in TEG-angle and MA occurred at platelet counts below 100×10^9 per l, corresponding to the counts which typically warrant clinical treatment.

There was a significant species difference between human and pig for both the lactated Ringer's and PAWB treatments for all the parameters except MA and G. The onset of clotting (TEG-R and TEG-TMRTG) is faster, the rate of clotting (K-time and angle) is faster, and the thrombin generation (MRTG) is greater in the pig than in the human with PAWB treatment.

Fibrinogen: lactated Ringer's and fibrinogen-adjusted whole blood

Similar to the relationship with platelet count, all of the TEG parameters showed a dose-related change (curvilinear component) with lactated Ringer's dilution when plotted against fibrinogen concentration (Fig. 6). While there was a significant dose-related change in all the TEG parameters with manipulation of fibrinogen, the slopes appeared to be flatter than the slope of the
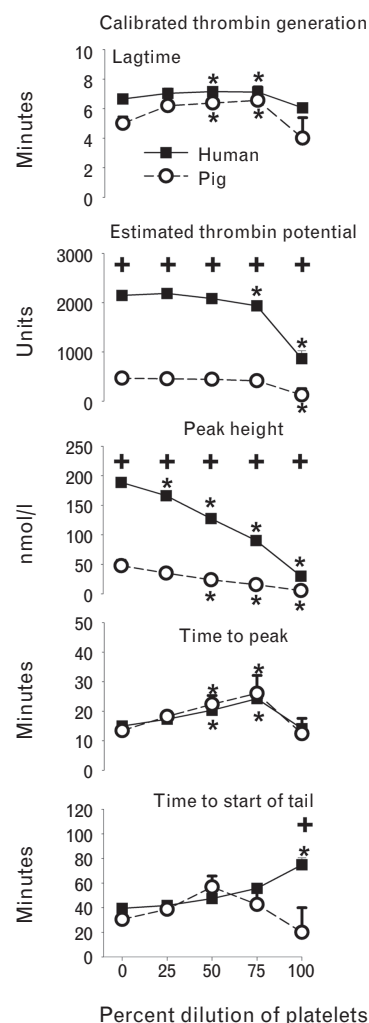
Fig. 3
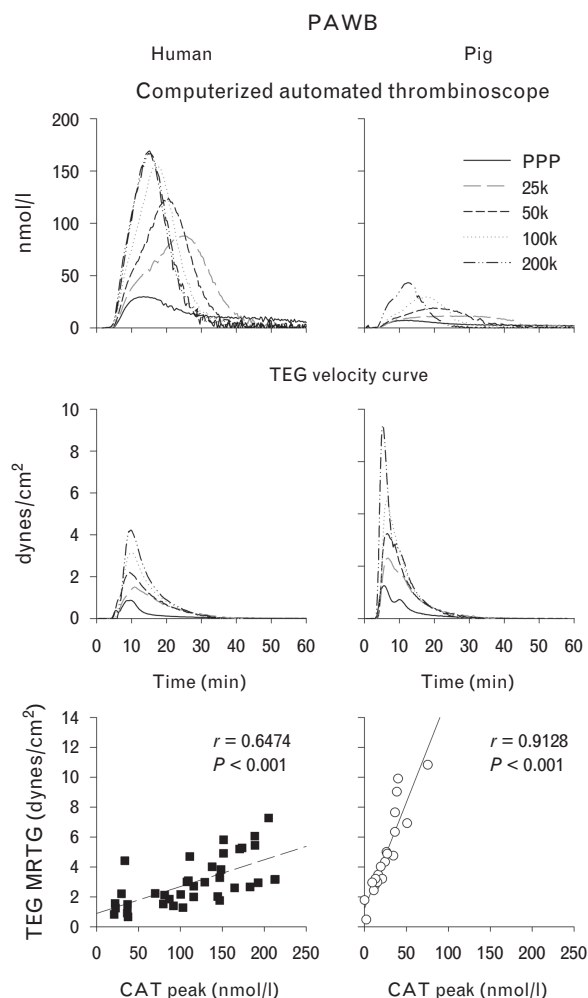
Comparison of the averaged computerized activated thrombinoscope (CAT) results (top two panels) and the velocity of the TEG curves (first derivative of the positive half of the curve, middle two panels) for the platelet-adjusted whole blood (PAWB) group for the humans on the left two upper panels and for the pigs on the right two upper panels. PPP = platelet poor plasma, the legend depicts the platelet count × 10^9 per l for each of the lines. A linear regression was performed comparing the individual values of the TEG- **MRTG** to the CAT peak height are shown in the bottom two panels, for the human (left bottom) and pig (right bottom). The correlation coefficient for each line was significant.

Fig. 4

Comparison between humans and pigs of CAT standard parameters for the platelet-adjusted whole blood (PAWB) group plotted against percentage dilution of the platelets. Symbols (*, +) as described for Fig. 1.

lactated Ringer's treatment expressed against fibrinogen concentration; although this comparison was not tested due to design of the study as mentioned above for the PAWB treatment. A possible reason for the flatness of the slope may be due to the fact that we could not reduce the fibrinogen concentration below that in the cryo-poor plasma that was used to dilute the cryo-rich plasma (see methods). Of note, however, is that even though the range of fibrinogen concentration (100–400 mg/dl) achieved in this study is not considered to be pathologically low, there were significant changes in all of the TEG parameters.

All of the parameters resulted in a statistically significant species difference [pig faster (TEG-R, TEG-K, TEG-angle, TEG-MRTG, TEG-TMRTG) or stronger (TEG-MA, TEG-G, TEG-TG) than human]. All TEG parameters with lactated Ringer's dilution had a significant interaction effect, indicating that the responses between the two species were not parallel with lactated Ringer's dilution while they were parallel in the FAWB treatment for TEG-MA, TEG-G, TEG-TG, and TEG-MRTG parameters.

Hematocrit: lactated Ringer's and hematocrit-adjusted whole blood procedure
Similar to the relationship with platelet count and fibrinogen concentration, all of the TEG parameters

Fig. 5

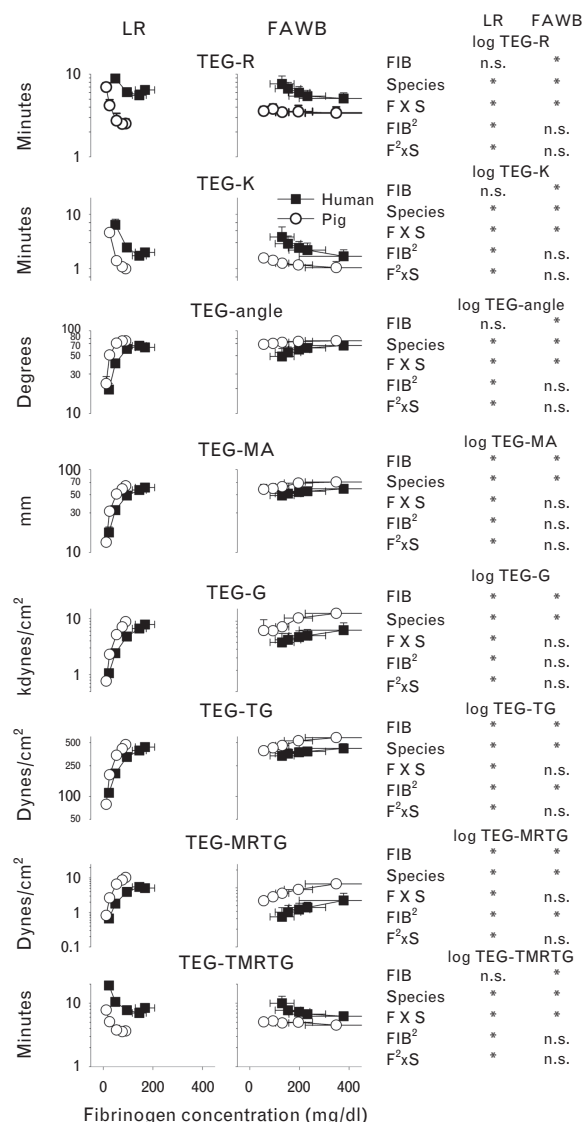Comparison between human and pig of the TEG parameters (log scale) plotted against hematocrit for lactated Ringer's (LR) dilution and hematocrit-adjusted whole blood (HAWB).* $P < 0.05$ blood component factor, species factor, or interaction effect, both linear and quadratic components. n.s. = not significant. HCT = H = hematocrit. S = species. $HCT^2 = H^2$ = square term in polynomial regression equation.

Statistical table (Fig. 5):

	LR	PAWB
log TEG-R		
PLT	*	n.s.
Species	*	*
P X S	n.s.	n.s.
PLT^2	*	n.s.
P^2xS	*	n.s.
log TEG-K		
PLT	*	*
Species	*	*
P X S	n.s.	n.s.
PLT^2	*	*
P^2xS	n.s.	n.s.
log TEG-angle		
PLT	*	*
Species	*	*
P X S	n.s.	n.s.
PLT^2	*	n.s.
P^2xS	n.s.	n.s.
log TEG-MA		
PLT	*	*
Species	n.s.	*
P X S	n.s.	*
PLT^2	*	*
P^2xS	*	n.s.
log TEG-G		
PLT	*	*
Species	n.s.	n.s.
P X S	n.s.	*
PLT^2	*	*
P^2xS	n.s.	n.s.
log TEG-TG		
PLT	*	*
Species	*	*
P X S	n.s.	*
PLT^2	*	*
P^2xS	*	n.s.
log TEG-MRTG		
PLT	*	*
Species	n.s.	*
P X S	n.s.	n.s.
PLT^2	*	*
P^2xS	*	n.s.
log TEG-TMRTG		
PLT	*	n.s.
Species	*	*
P X S	n.s.	n.s.
PLT^2	*	n.s.
P^2xS	*	n.s.

X-axis: Platelet count (10^9/l)

Fig. 6

Comparison between human and pig of the TEG parameters (log scale) plotted against fibrinogen concentration for lactated Ringer's (LR) dilution and fibrinogen-adjusted whole blood (FAWB).* $P < 0.05$ blood component factor, species factor, or interaction effect, both linear and quadratic components. n.s. = not significant. FIB = F = fibrinogen. S = species. $FIB^2 = F^2$ = square term in polynomial regression equation.

Statistical table (Fig. 6):

	LR	FAWB
log TEG-R		
FIB	n.s.	*
Species	*	*
F X S	*	*
FIB^2	*	n.s.
F^2xS	*	n.s.
log TEG-K		
FIB	n.s.	*
Species	*	*
F X S	*	*
FIB^2	*	n.s.
F^2xS	*	n.s.
log TEG-angle		
FIB	n.s.	*
Species	*	*
F X S	*	*
FIB^2	*	n.s.
F^2xS	*	n.s.
log TEG-MA		
FIB	*	*
Species	*	*
F X S	*	n.s.
FIB^2	*	n.s.
F^2xS	*	n.s.
log TEG-G		
FIB	*	*
Species	*	*
F X S	*	n.s.
FIB^2	*	n.s.
F^2xS	*	n.s.
log TEG-TG		
FIB	*	*
Species	*	*
F X S	*	n.s.
FIB^2	*	*
F^2xS	*	n.s.
log TEG-MRTG		
FIB	*	*
Species	*	*
F X S	*	n.s.
FIB^2	*	*
F^2xS	*	n.s.
log TEG-TMRTG		
FIB	n.s.	*
Species	*	*
F X S	*	*
FIB^2	*	n.s.
F^2xS	*	n.s.

X-axis: Fibrinogen concentration (mg/dl)

showed a dose-related change with lactated Ringer's dilution when plotted against hematocrit (Fig. 7). Interestingly, all of the TEG parameters except TEG-R and TEG-TMRTG also were significantly related to the dilution of the red blood cells, but the relationship was in the opposite direction to that with dilution with lactated Ringer's, suggesting that increasing the number of red blood cells may interfere with the rate of clot formation and the strength of the clot under the in-vitro conditions of low shear.

There was a significant species effect of all the TEG parameters except TEG-MA, TEG-G and TEG-TG with lactated Ringer's dilution when expressed per change in hematocrit; the porcine TEG-R, TEG-K, TEG-TMRTG, and TEG-velocity were faster than that of the human, and the TEG-angle and TEG-MRTG were greater in the pig than the human. For the HAWB treatment, the response of the pig was significantly different from that of the human for the TEG-R, TEG-K, TEG-angle, TEG-MA, TEG-G, and
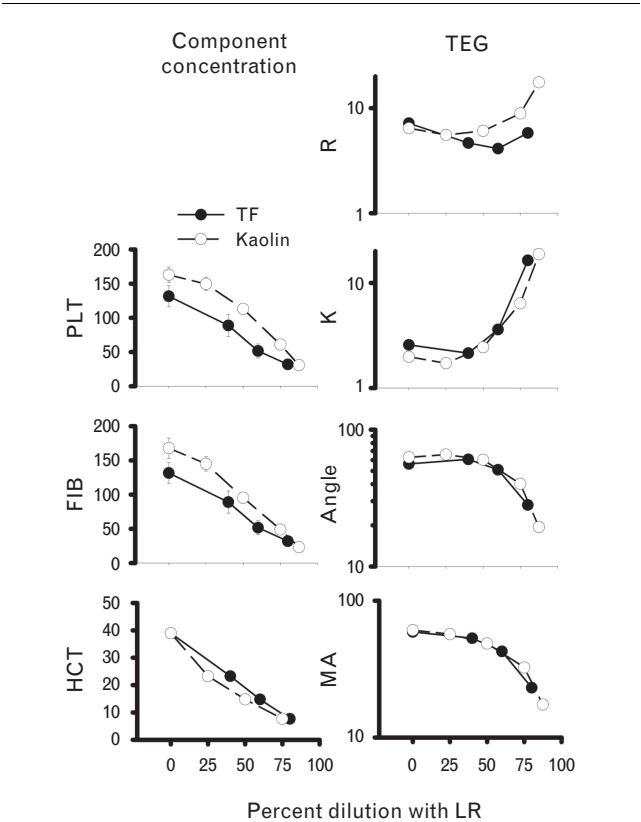
Fig. 7
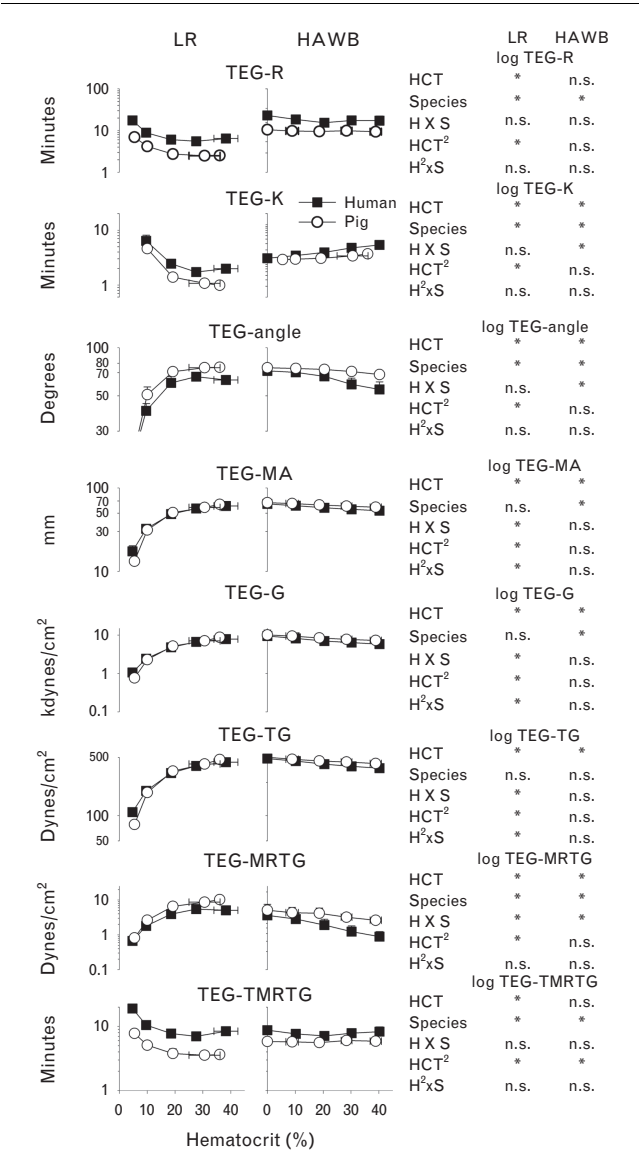
Comparison between human and pig of the TEG parameters (log scale) plotted against hematocrit for lactated Ringer's (LR) dilution and hematocrit-adjusted whole blood (HAWB). * $P < 0.05$ blood component factor, species factor, or interaction effect, both linear and quadratic components. n.s. = not significant. HCT = H = hematocrit. S = species. HCT^2 = H^2 = square term in polynomial regression equation.

Fig. 8

Component concentrations and TEG parameters in human samples comparing the use of Tissue Factor (TF; Innovin = 1 : 500 dilution) with Kaolin as the accelerants. * $P < 0.05$ accelerant, dilution, or interaction effect, both linear and quadratic components. n.s. = not significant. A = accelerant. D = dilution. Data from the TF samples come from Darlington et al. [8].

TEG-MRTG, and the TEG-TMRTG was faster in the pig than in the human.

Comparison of kaolin versus tissue factor (Innovin) as the accelerant for thrombelastography

Previous work from our laboratory [19] showed the effect of lactated Ringer's dilution on the TEG, but it was performed using TF (Innovin = 1 : 500 dilution) as the accelerant. As the TEGs we have previously published for pigs [20] used kaolin as an accelerant, we decided to

use it for the humans as well. As there is some controversy as to which accelerant should be used, we compared the results we had for the lactated Ringer's dilution of human samples with those from the Darlington et al. study [19]. As seen in Fig. 8, there were no significant differences in the TEG-K, TEG-angle, and TEG-MA responses to lactated Ringer's dilution between kaolin and TF, although there was a difference in TEG-R between the accelerants, especially at the higher dilutions.

Visual appearance of thrombelastography tracings

The TEG manual [11] states that the pattern of the TEG tracing can be used to qualitatively analyze the coagulation status of the blood. The coordinates of each of the dilutions in each of the treatments were averaged, and their thromboelastograms are shown in Fig. 9, for both the human and pig. No statistical analysis was performed on these curves. Qualitatively, the curves for the pig appear to have a steeper angle and a slightly larger MA than the human. Also, the change in each of the treatments with dilution of each component or with LR

Fig. 9
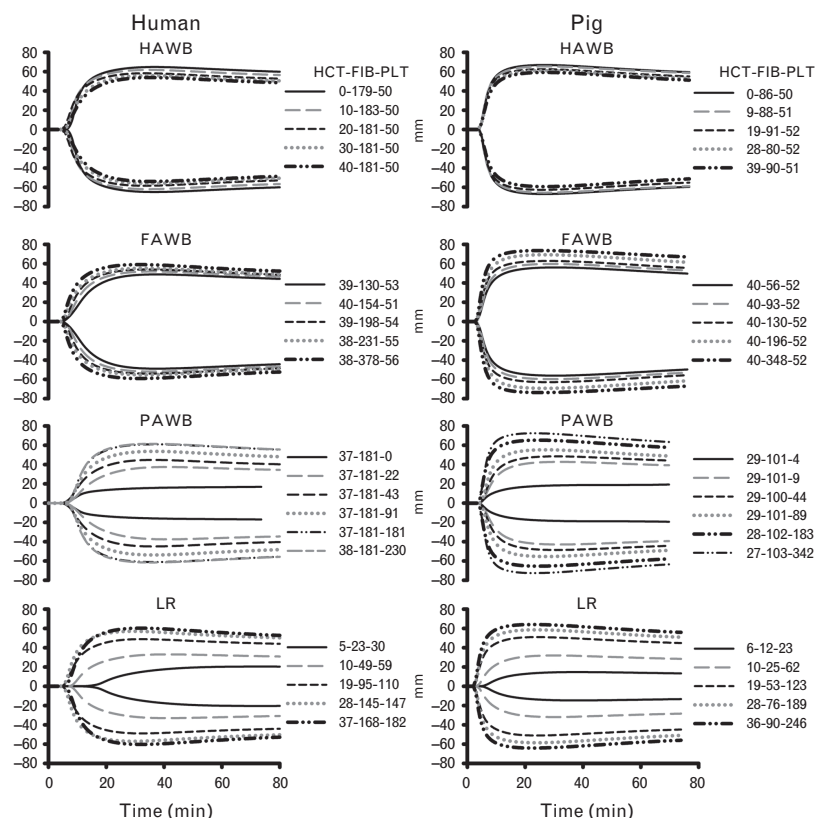
The TEG curves averaged for each of the specific component manipulations compared with lactated Ringer's (LR) treatment in which all components are diluted simultaneously. The human curves are on the left, and the pig curves are on the right. Numbers to right of each panel are the corresponding hematocrit (HCT) in %RBC, fibrinogen (FIB) in mg/dl, and platelet count (PLT) in ($\times10^9$/l).

appear to be similar between human and pig. Manipulation of platelet counts yields TEG tracings that are indistinguishable from those obtained by whole blood dilution using lactated Ringer's and suggests that platelets are the driving force. The changes in the TEG tracings obtained by manipulation of either fibrinogen or hematocrit are subtle suggesting that the hematocrit has little influence on TEG and the levels of fibrinogen were not deficient to affect the TEG.

Discussion

By changing hematocrit, platelet count, or plasma composition one component at a time, we demonstrated that the major factor that affected the MA and angle was the platelet count. In fact, changing platelets alone resulted in TEG profiles and parameters that were qualitatively similar to those brought about by lactated Ringer's dilution. Thus, superficially similar TEG tracing patterns can be produced by divergent mechanisms. These results may be pertinent to the potential use of the TEG for goal-directed resuscitation therapy of trauma patients [22]. This may limit the interpretation of TEG in acute care settings, particularly by inexperienced personnel. TEG

visual output should be correlated with numerical parameters and other relevant clinical data in guiding patient care.

We also showed that the response of swine blood was parallel to that of human blood, although there were offsets especially of TEG-R (with all components) and TEG-angle (with fibrinogen) that confirmed that the swine are hypercoagulable compared with humans.

The method that we used to manipulate each component by using each patient's own blood, was the ideal way to assess the effect of specific changes in blood composition on the TEG responses. We were interested in determining whether the TEG could be used to specifically determine which blood component was deficient, as is sometimes claimed for use of the TEG in determining goal directed therapy [12]. There have been several clinical studies, which corroborate the poor accuracy of the ROTEM for predicting specific hypofibrinogenia or thrombocytopenia needs [23,24].

Reduction in HCT does not result in decreased blood coagulability as measured by TEG parameters, but rather

appears to increase MA and angle. It is possible that the increased viscosity imparted by red blood cells impedes clot formation in the TEG system. The relevance of this observation to in-vivo or ex-vivo clot formation is unclear, although Roeloffzen *et al.* [25] showed that RBC transfusion in anemic humans reduced MA. A similar in-vitro test [17] found the MA to be reduced with increasing HCT, however interpretation of their results is limited as they simultaneously reduced plasma as well as hematocrit.

Decreases in MA and angle are detectable across a range of platelet counts that includes part of the normal range. Changes in TEG parameters are even more pronounced across a range of platelet counts $(50-100 \times 10^9$ per l) that frequently do not prompt platelet transfusion [26]. These findings suggest that bleeding patients may derive clinical benefit (i.e., increased clot strength and rate of clot formation) from platelet transfusion even if platelet counts are greater than 100×10^9 per l. Prospective studies are required to confirm this possibility and define optimal platelet counts for various clinical scenarios.

Clot strength (MA) and rate of clot formation (R time and angle) increase as fibrinogen increases over a range of $100-400$ mg/dl, which includes the normal range for humans. In clinical practice, fibrinogen-enriched cryoprecipitate is not typically transfused in bleeding patients until fibrinogen levels drop below 100 mg/dl. Our findings suggest that bleeding patients may derive clinical benefit from cryoprecipitate (or fibrinogen) transfusion even if fibrinogen levels are greater than 100 mg/dl. Prospective studies are required to confirm this possibility and define optimal fibrinogen levels for various clinical scenarios.

Dilution of whole blood with lactated Ringer's results in profound hypocoagulability as measured by TEG, particularly at greater than or equal to 50% dilution, a level which can plausibly be reached in the setting of hemorrhage resuscitated with large volumes of asanguineous crystalloid or colloid.

In conclusion, superficially similar TEG tracing patterns can be produced by divergent mechanisms. This may limit the interpretation of TEG in acute care settings, particularly by inexperienced personnel. TEG visual output should be correlated with numerical parameters and other relevant clinical data in guiding patient care. As similar responses to human blood were seen in pig blood, studies of blood components used in hemostatic resuscitation (as part of damage control resuscitation strategy) in swine hemorrhage models should be valid.

Acknowledgements

Conflicts of interest

The authors declare that they have no conflicts of interest.

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